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One-pot green synthesis and bio-assay of pyrazolylphosphonates

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Abstract An efficient one-pot synthesis of bioactive pyrazolylphosphonates is accomplished by the reaction of 3-methyl-1-phenyl-2-pyrazoline-5-one with various aryl aldehydes and diethyl phosphite in the presence of β-cyclodextrin (β-CD) as a catalyst. The compounds were tested for their anti-oxidant activity by DPPH and H_2O_2 radical scavenging assay and for anticancer activity against breast cancer (MCF-7), prostate cancer (DU-145) and lung cancer (A-549) cell lines with sulforodamine-B (SRB) assay. Most of the synthesized compounds showed promising anti-oxidant activity and significant anticancer activity when compared with the standard drugs.

Keyword Pyrazolylphosphonates · Green synthesis · β-cyclodextrin · Anti-oxidant activity · Anticancer activity

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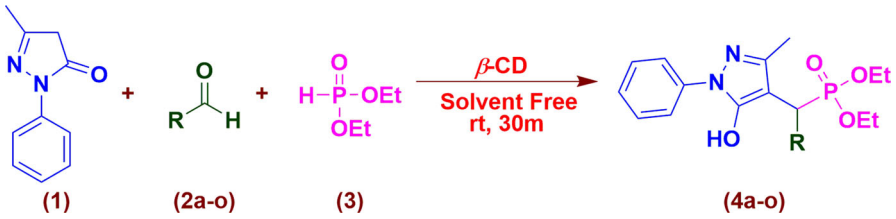
Introduction

In recent years, significant effort has been devoted to the synthetic studies of organophosphorus compounds since they have several biological properties include antitumor [1], anti-oxidant [2], antimicrobial [3], anticoagulant [4], anti-inflammatory [5], antiviral [6], enzyme inhibition [7], plant growth regulation, herbicidal and pesticidal activities [8–10]. Owing to their potential bioactivity, low toxicity and the ease of substitution with conventional heterocyclic groups, phosphoryl compounds are perfect substrates for use in drug design. The molecular modifications involving the introduction of organophosphorus functionalities in simple synthons is a very promising route for the preparation of the new generation of biologically active phosphoryl compounds [11].

Pyrazole and its derivatives are a class of important aza-heterocyclic compounds [12] with a variety of bioactivities such as antimicrobial [13], anticancer [14], anticonvulsant [15], analgesic, antipyretic, anti-inflammatory [16], antibacterial [17], antifungal [18], CNS regulating [19], enzyme inhibition [20], hypoglycemic, anti-oxidant [21] and antihypertensive [22] activities.

Due to the tremendous synthetic and bio-potent value of phosphoryl and pyrazole motifs, a variety of pyrazolylphosphonates (**4a–o**) are synthesized by the condensation of 3-methyl-1-phenyl-2-pyrazoline-5-one (**1**) with various aryl aldehydes (**2a–o**) and diethyl phosphite (**3**), expecting them to have good bioactivities. However, very few synthetic protocols are available for the syntheses of pyrazolylphosphonates by a three-component reaction of pyrazolones, with various aryl aldehydes and dialkyl phosphite [23–25]. They have drawbacks such as long reaction times, requirement of stoichiometric quantities of toxic catalysts, low product yields and generation of wastage. Thus, there remains a need for novel and improved synthetic routes that are elegant, inexpensive and environmentally benign.

The art of performing green and efficient chemical transformations involves non-toxic reaction conditions. Cyclic oligosaccharides possessing hydrophobic cavities are well known as supramolecular catalysts, which, by reversible formation of host–guest complexes, activate the organic molecules and thus efficiently catalyze the reactions under green conditions [26]. β-CD is the example of these biomimetic catalysts used extensively in many novel transformations with high yield and good selectivity, includes the synthesis of important classes of biologically active heterocycles like pyrroles [27], thiazoles [28], quinolones [29], quinazolines [30], and oxindoles [31]. In the present work, a green procedure for the synthesis of pyrazolylphosphonates (**4a–o**) catalyzed by β-CD in solvent-free conditions has been reported (Scheme 1). The title compounds were screened for their anti-oxidant activity by DPPH and H_2O_2 radical scavenging assay and for anticancer activity against breast cancer (MCF-7), prostate cancer (DU-145) and lung cancer (A-549) cell lines with sulfarodamine-B (SRB) assay.



Scheme 1 Synthesis of diethyl((5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)(aryl)methyl) phosphonates(**4a–o**)

Experimental

Analysis and instruments

All the required chemicals were purchased from Sigma Aldrich and the solvents from Merck and were used without further purification. The completion and purity of the reactions were monitored by TLC, performed on silica gel aluminum 60 F-254 thin layer plates procured from Merck, and visualization on TLC was achieved by UV light and iodine indicator. Melting points of the compounds were determined on Guna digital melting point apparatus using open capillary tubes and are uncorrected. Infrared spectra were recorded on FT-IR Bruker ALPHA Interferometer and wave numbers are given in cm^{-1}. NMR spectra were recorded on a Bruker instrument operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C and 161.9 MHz for ^{31}P in $CDCl_3$. TMS was used as an internal standard. Assignments of the signals are based on the chemical shifts and intensity patterns. Chemical shift (δ) and coupling constant (J) are expressed in ppm and Hz, respectively. Mass spectra were recorded on a LC–MS/MS-TOF API QSTAR PULSAR spectrometer; samples were introduced by the infusion method using the electrospray ionization technique (ESI).

General procedure for synthesis of pyrazolylphosphonate derivatives (4a–o)

To a mixture of 3-methyl-1-phenyl-2-pyrazoline-5-one (1.0 mmol), aryl aldehydes (1.2 mmol), and diethylphosphite (2.0 mmol) were added the β-CD (10 mol%) in a round-bottomed flask. The reaction mixture was stirred at room temperature under neat conditions. The progress of the reaction was indicated with observation of new spots in the TLC after 10 min. The reaction was run with stirring of the reaction mixture for 30 min. After completion of the reaction, the reaction mixture was poured into cold water and extracted three times with ethyl acetate (3 5 mL). The organic layer was washed with 10% $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered and concentrated in a rota-evaporator to leave crude product which was purified by recrystallization from ethanol. The aqueous layer was frozen to 0–10 °C to precipitate β-CD as a white solid. This was collected by filtration, washed with water and dried for reuse.

Diethyl((4-ethoxyphenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate(**4a**) Brown solid; Yield: 96%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *ν* 3382 (–OH), 2968 (Ar–H), 1240 (–P=O), 1027 (–P–O–C–), 746 (–P–C–). ^{1}H-NMR ($CDCl_3$): *δ* 7.80 (2H, d, *J* = 8.0 Hz), 7.40 (2H, t, *J* = 12.0 Hz), 7.32 (1H, t, *J* = 12.0 Hz), 7.11 (2H, d, *J* = 8.0 Hz), 6.84 (2H, d, *J* = 8.0 Hz), 5.91 (1H, s), 4.90 (1H, d, *J* = 8.0 Hz), 4.14-3.97 (4H, m), 2.28 (3H, s), 1.40 (3H, t, *J* = 12.0 Hz), 1.33 (3H, t, *J* = 12.0 Hz), 1.21 (3H, t, *J* = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): *δ* 164.13, 158.40 (d, *J*c-p = 12.0 Hz), 147.81 (d, *J*c-p = 52.0 Hz), 138.96, 130.04, 129.98, 128.71(d, *J*c-p = 52.0 Hz), 121.77, 114.75, 94.25, (d, *J*c-p = 16.0 Hz), 64.40 (d, *J*c-p = 36.0 Hz), 50.49 (d, *J*c-p = 140.0 Hz), 16.44, 14.82, 12.65. ^{31}P-NMR ($CDCl_3$): *δ* 18.58. MS (ESI): *m/z* 445.18 Anal. Calcd. for $C_{23}H_{29}N_2O_5P$: C, 62.15; H, 6.58; N, 6.30; O, 18.00; P, 6.97. Found: C, 62.06; H, 6.43; N, 6.22; O, 17.91; P, 6.88.

Diethyl((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(p-tolyl)methyl)phosphonate(**4b**) [24] Yellow solid; Yield: 96%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *ν* 3404 (–OH), 2980 (Ar–H), 1247 (–P=O), 1024 (–P–O–C–), 756 (–P–C–). ^{1}H-NMR ($CDCl_3$): *δ* 7.74 (2H, d, *J* = 8.0 Hz), 7.38 (2H, t, *J* = 12.0 Hz), 7.24 (1H, t, *J* = 12.0 Hz), 7.08 (2H, d, *J* = 8.0 Hz), 6.82 (2H, d, *J* = 8.0 Hz), 5.76 (1H, s), 4.98 (1H, d, *J* = 10.0 Hz), 4.17-3.88 (4H, m), 2.24 (3H, s), 1.39 (3H, t, *J* = 12.0 Hz), 1.24 (3H, t, *J* = 12.0 Hz), 1.16 (3H, t, *J* = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): *δ* 166.18, 159.42 (d, *J*c-p = 12.0 Hz), 148.76 (d, *J*c-p = 48.0 Hz), 139.96, 130.08, 127.58, 126.72(d, *J*c-p = 54.0 Hz), 122.71, 112.78, 96.22, (d, *J*c-p = 24.0 Hz), 64.42 (d, *J*c-p = 48.0 Hz), 52.42 (d, *J*c-p = 128.0 Hz), 16.42, 14.81, 12.68. ^{31}P-NMR ($CDCl_3$): *δ* 20.45. MS (ESI): *m/z* 415.17 Anal.Calcd. for $C_{22}H_{27}N_2O_4P$: C, 63.76; H, 6.57; N, 6.76; O, 15.44; P, 7.47. Found: C, 63.69; H, 6.50; N, 6.68; O, 15.34; P, 7.30.

Diethyl((3,4-dimethoxyphenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate(**4c**) Brown solid; Yield: 96%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *ν* 3397 (–OH), 2980 (Ar–H), 1264 (–P=O), 1014 (–P–O–C–), 752 (–P–C–). ^{1}H-NMR ($CDCl_3$): *δ* 7.77 (2H, d, *J* = 8.0 Hz), 7.73 (2H, t, *J* = 8.0 Hz), 7.37 (2H, t, *J* = 12.0 Hz), 6.69 (1H, d, *J* = 8.0 Hz), 6.62 (1H, d, *J* = 8.0 Hz), 6.55 (1H, d, *J* = 8.0 Hz), 5.98 (1H, s), 4.94 (1H, d, *J* = 10.0 Hz), 3.90-3.72 (4H, m), 2.90 (3H, s), 2.83 (3H, s), 2.34 (3H, s), 1.27 (3H, t, *J* = 12.0 Hz), 1.22 (3H, t, *J* = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): *δ* 162.33, 153.60, 151.07, 148.75, 14.31, 146.24, 138.48, 137.66, 130.44, 128.79, 126.83, 124.82, 121.10, 119.37, 115.58, 110.72 (d, *J*c-p = 172.0 Hz), 64.23 (d, *J*c-p = 156.0 Hz), 61.38, 56.31 (d, *J*c-p = 184.0 Hz), 16.54, 13.36. ^{31}P-NMR ($CDCl_3$): *δ* 19.57. MS (ESI): *m/z* 461.23 Anal. Calcd. for $C_{23}H_{29}N_2O_6P$: C, 59.99; H, 6.35; N, 6.08; O, 20.85; P, 6.73 Found: C, 59.87; H, 6.26; N, 5.97; O, 20.76; P, 6.64.

Diethyl((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(3,4,5-trimethoxyphenyl)methyl)phosphonate(**4d**) Yellow solid; Yield: 96%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *ν* 3396 (–OH), 2982 (Ar–H), 1256 (–P=O), 1033 (–P–O–C–), 757 (–P–C–). ^{1}H-NMR ($CDCl_3$): *δ* 7.76 (2H, d, *J* = 8.0 Hz), 7.38 (2H, t, *J* = 12.0 Hz), 7.20 (1H, t, *J* = 12.0 Hz), 6.94 (1H, s), 6.78 (1H, s), 5.90 (1H, s), 4.90 (1H, d, *J* = 12.0 Hz), 4.18–3.99 (4H, m), 3.84–3.73 (9H, m), 2.89 (3H, s), 1.22 (3H, t, *J* = 12.0 Hz), 1.15 (3H, t, *J* = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): *δ* 164.13, 158.40 (d, *J*c-p = 12.0 Hz), 147.81 (d, *J*c-p = 52.0 Hz), 138.96, 131.23 (d, *J*c-p = 24.0 Hz), 128.78, 128.57, 121.77, 114.76 (d, *J*c-p = 12.0 Hz), 94.25 (d, *J*c-p = 16.0 Hz), 64.44, 63.47 (d, *J*c-p = 24.0 Hz), 61.90 (d, *J*c-p = 24.0 Hz), 45.33

(d, *J*c-p = 244.0 Hz), 16.44, 14.82, 12.65. ^{31}P-NMR ($CDCl_3$): δ 21.29. MS (ESI): *m/z* 491.90 Anal. Calcd. for $C_{24}H_{31}N_2O_7P$: C, 58.77; H, 6.37; N, 5.71; O, 22.83; P, 6.31 Found: C, 58.70; H, 6.31; N, 5.64; O, 22.74; P, 6.24.

Diethyl((4-(diethylamino)phenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl) phosphonate(**4e**) Brown solid; Yield: 96%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *v* 3397 (–OH), 2924 (Ar–H), 1245 (–P=O), 1018 (–P–O–C–), 742 (–P–C–). ^{1}H-NMR ($CDCl_3$): 7.56 (2H, d, J = 8.0 Hz), 7.52 (2H, t, J = 12.0 Hz), 7.46 (1H, m), 7.02 (1H, d, J = 8.0 Hz), 6.96 (2H, d, J = 8.0 Hz), δ 5.82 (1H, s), 4.76 (1H, d, J = 8.0 Hz), 4.14–3.36 (4H, m), 3.60–3.56 (2H, m), 3.26–3.22 (2H, m), 2.64–2.60 (2H, m), 2.48 (3H, s), 1.97 (3H, s), 1.32 (6H, m), 1.20 (3H, t, J = 12.0 Hz), 1.06 (3H, t, J = 12.0 Hz). ^{13}C-NMR ($CDCl_3$) δ 151.42, 147.46, 145.61 (d, *J*c-p = 54.0 Hz), (d, *J*c-p = 56.0 Hz), 138.24, 130.59 (d, *J*c-p = 68.0 Hz), 129.81, 126.75, 122.40, 111.56 (d, *J*c-p = 76.0 Hz), 97.11 (d, *J*c-p = 7.0 Hz), 64.08 (d, *J*c-p = 84.0 Hz), 46.32 (d, *J*c-p = 186.0 Hz), 44.48, 16.42 (d, *J*c-p = 20.0 Hz), 13.81 (d, *J*c-p = 84.0 Hz), 13.26. ^{31}P-NMR ($CDCl_3$): δ 21.48. MS (ESI): *m/z* 472.68 Anal. Calcd. for $C_{25}H_{34}N_3O_4P$: C, 63.68; H, 7.27; N, 8.91; O, 13.57; P, 6.57. Found: C, 63.62; H, 7.16; N, 8.86; O, 13.45; P, 6.49.

Diethyl((3-fluorophenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate(**4f**) Brown solid; Yield: 91%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *v* 3386 (–OH), 2980 (Ar–H), 1225(–P=O), 1031 (–P–O–C–), 751 (–P–C–). ^{1}H-NMR ($CDCl_3$): δ 7.60 (2H, d, J = 8.0 Hz), 7.34 (2H, t, J = 12.0 Hz), 7.10 (1H, d, J = 8.0 Hz), 6.96 (1H, d, J = 8.0 Hz), 6.0 (1H, d, J = 8.0 Hz), 6.75 (1H, m), 5.88 (1H, s), 4.90 (1H, d, J = 16.0 Hz), 3.95–3.72 (4H, m), 2.76 (3H, s), 1.16 (3H, t, J = 12.0 Hz), 1.12 (3H, t, J = 12,0 Hz). ^{13}C-NMR ($CDCl_3$): δ 164.13, 156.96, 147.73 (d, *J*c-p = 48.0 Hz), 146.37, 138.67, 137.83, 128.76 (d, *J*c-p = 20.0 Hz), 125.66, 123.15, 121.39, 118.6, 114.36. (d, *J*c-p = 88.0 Hz) 112.87 (d, *J*c-p = 84.0 Hz), 104.25, 93.46, 63.56 (d, *J*c-p = 78.0 Hz), 44.44 (d, *J*c-p = 144.0 Hz), 16.31 12.59, 11.92. ^{31}P-NMR ($CDCl_3$): δ 18.99. MS (ESI): *m/z* 419.05 Anal.Calcd. for $C_{21}H_{24}FN_2O_4P$: C, 60.28; H, 5.78; F, 4.54; N, 6.70; O, 15.30; P, 7.40. Found: C, 60.19; H, 5.71; F, 4.46; N, 6.64; O, 15.22; P, 7.31.

Diethyl((4-chlorophenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate(**4g**) [23] White solid; Yield: 96% M.p. > 300 °C. FT-IR (neat, cm^{-1}) *v* 3416 (–OH), 2992 (Ar–H), 1253 (–P=O), 1032 (–P–O–C–), 748 (–P–C–). ^{1}H-NMR ($CDCl_3$): δ 7.86 (2H, d, J = 8.0 Hz), 7.54 (2H, t, J = 12.0 Hz), 7.28 (2H, t, J = 12.0 Hz), 7.16 (1H, m), 7.08 (2H, m), 5.87 (1H, s), 4.84 (1H, d, J = 12.0 Hz), 4.08–3.76 (4H, m), 2.24 (3H, s), 1.38 (3H, t, J = 12.0 Hz), 1.26 (3H, t, J = 12.0 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 156.78, 146.90, 143.25, 138.95, 1136.23, 131.55 (d, *J*c-p = 78.0 Hz), 130.68 (d, *J*c-p = 74.0 Hz), 129.69, 128.64 (d, *J*c-p = 12.0 Hz), 121.78 (d, *J*c-p = 54.0 Hz), 119.21, 103.30, 63.24 (d, *J*c-p = 76.0 Hz), 61.62 (d, *J*c-p = 48.0 Hz), 44.93 (d, *J*c-p = 184.0 Hz), 16.68 14.33, 12.58. ^{31}P-NMR ($CDCl_3$): δ 20.28. MS (ESI): *m/z* 439.12. Anal.Calcd. for $C_{21}H_{24}ClN_2O_4P$: C, 58.00; H, 5.56; Cl, 8.15; N, 6.44; O, 14.72; P, 7.12. Found: C, 57.95; H, 5.48; Cl, 8.04; N, 6.32; O, 14.68; P, 7.06.

Diethyl((4-bromophenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate(**4h**) Brown solid. Yield: 92%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *v* 3397 (-OH), 2980 (Ar–H), 1214 (–P=O), 1072 (–P–O–C–), 752 (–P–C–). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.74 (2H, d, J = 8.0 Hz), 7.45 (2H, t, J = 12.0 Hz),

7.32 (2H, t, J = 12.0 Hz), 7.15(1H, m), 7.13 (2H, m), 5.93 (1H, s), 4.93 (1H, d, J = 12.0 Hz), 4.15-3.99 (4H, m), 2.28 (3H, s), 1.36 (3H, t, J = 12.0 Hz), 1.24 (3H, t, J = 12.0 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 155.80, 146.90, 143.25, 138.95, 1136.23, 131.55 (d, Jc-p = 78.0 Hz), 130.63 (d, Jc-p = 74.0 Hz), 129.69, 128.76 (d, Jc-p = 12.0 Hz), 121.76 (d, Jc-p = 54.0 Hz), 119.27, 103.37, 63.28 (d, Jc-p = 76.0 Hz), 61.86 (d, Jc-p = 48.0 Hz), 44.59 (d, Jc-p = 228.0 Hz), 16.42 14.37, 12.53. ^{31}P-NMR ($CDCl_3$): δ 21.78. MS (ESI): *m/z* 480.05 Anal. Calcd. for $C_{21}H_{24}BrN_2O_4P$: C, 52.62; H, 5.05; Br, 16.67; N, 5.84; O, 13.35; P, 6.46. Found: C, 52.54; H, 4.94; Br, 16.56; N, 5.78; O, 13.22; P, 6.38.

Diethyl((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(4-nitrophenyl)methyl)phosphonate(**4i**) [24, 25] Light yellow solid; Yield: 91%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) ν 3412 (–OH), 2996 (Ar–H), 1238 (–P=O), 1017 (–P–O–C–), 757 (–P–C–). ^{1}H-NMR ($CDCl_3$): δ 7.86 (2H, d, J = 8.0 Hz), 7.42 (2H, t, J = 12.0 Hz), 7.38 (1H, t, J = 12.0 Hz), 7.06 (2H, d, J = 8.0 Hz), 6.90 (2H, d, J = 8.0 Hz), 5.88 (1H, s), 4.84 (1H, d, J = 12.0 Hz), 4.04–3.86 (4H, m), 2.56 (3H, s), 1.34 (3H, t, J = 12.0 Hz), 1.16 (3H, t, J = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 164.18, 158.57 (d, Jc-p = 12.0 Hz), 146.23 (d, Jc-p = 52.0 Hz), 137.96, 134.82, 123.36, 128.76 (d, Jc-p = 52.0 Hz), 121.88, 114.64, 94.34, (d, Jc-p = 24.0 Hz), 64.82 (d, Jc-p = 36.0 Hz), 48.46 (d, Jc-p = 136.0 Hz), 16.64, 14.24, 12.16. ^{31}P-NMR($CDCl_3$): δ 23.24. MS (ESI): *m/z* 446.14 Anal. Calcd. for $C_{21}H_{24}N_3O_6P$: C, 56.63; H, 5.43; N, 9.43; O, 21.55; P, 6.95 Found: C, 56.54; H, 5.33; N, 9.36; O, 21.48; P, 6.90.

Diethyl((5-chloro-2-fluorophenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate(**4j**) Yellow solid; Yield: 90%. M.p.: > 300 °C. FT-IR (neat, cm^{-1}) ν 3428 (–OH), 2987 (Ar–H), 1230 (–P=O), 1046 (–P–O–C–), 738 (–P–C–). ^{1}H-NMR ($CDCl_3$) δ 7.68 (2H, d, J = 8.0 Hz), 7.46 (2H, t, J = 12.0 Hz), 7.34 (1H, t, J = 12.0 Hz), 7.28 (1H, t, J = 12.0 Hz), 7.13 (1H, m), 6.84 (1H, m), 5.86 (1H, s), 4.88 (1H, d, J = 12.0 Hz), 4.07-3.76 (4H, m), 2.28 (3H, s), 1.36 (3H, t, J = 12.0 Hz), 1.16 (3H, t, J = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 158.32, 157.96, 144.82, (d, Jc-p = 14.0 Hz), 136.24, 132.56, 131.67 (d, Jc-p = 20.0 Hz), 128.68, 127.86 (d, Jc-p = 69.0 Hz), 125.94, 116.68 (d, Jc-p = 24.0 Hz), 63.68 (d, Jc-p = 52.0 Hz), 43.84 (d, Jc-p = 152.0 Hz), 16.48, 13.42, 11.56. ^{31}P-NMR ($CDCl_3$) δ 21.04. MS (ESI): *m/z* 453.11 Anal. Calcd. for $C_{21}H_{23}ClFN_2O_4P$: C, 55.70; H, 5.12; Cl, 7.83; F, 4.20; N, 6.19; O, 14.13; P, 6.84. Found: C, 55.62; H, 5.02; Cl, 7.76; F, 4.12; N, 6.10; O, 14.03; P, 6.76.

Diethyl((5-bromo-2-fluorophenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate(**4k**) Brown solid; Yield: 90%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) ν 3396 (–OH), 2978 (Ar–H), 1186 (–P=O), 1036 (–P–O–C–), 760 (–P–C–). ^{1}H-NMR ($CDCl_3$): δ 7.87 (1H, d, J = 8.0 Hz), 7.77 (1H, d, J = 8.0 Hz), 7.64 (2H, d, J = 8.0 Hz), 7.28 (2H, t, J = 8.0 Hz), 7.13 (1H, t, J = 8.0 Hz), 6.84 (1H, t, J = 12.0 Hz), 5.78 (1H, s), 5.27 (1H, d, J = 12.0 Hz), 4.07–3.82 (2H, m), 3.81–3.76 (1H, m), 3.74–3.56 (1H, m), 2.27 (3H, s), 1.32 (3H, t, J = 12 Hz), 1.20 (3H, t, J = 12 Hz). ^{13}C-NMR ($CDCl_3$): δ 160.35, 157.91, 145.81, (d, Jc-p = 24.0 Hz), 137.13, 132.04, 131.64 (d, Jc-p = 60.0 Hz), 128.74, 127.84 (d, Jc-p = 56.0 Hz), 125.96, 116.68 (d, Jc-p = 78.0 Hz), 63.64, 43.65 (d, Jc-p = 184.0 Hz), 16.24, 13.48, 11.50. ^{31}P-NMR ($CDCl_3$): δ 18.19. MS (ESI): *m/z* 497.06 Anal. Calcd. for C, 50.72; H, 4.66; Br, 16.07; F, 3.82; N, 5.63; O, 12.87; P, 6.23. Found: C, 50.64; H, 4.58; Br, 16.00; F, 3.74; N, 5.52; O, 12.78; P, 6.15.

Diethyl((4-chloro-3-nitrophenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl) phosphonate(**4l**) White solid; Yield: 88%. M.p. > 300 °C, FT-IR (neat, cm^{-1}) *ν* 3425 (–OH), 2979 (Ar–H), 1246 (–P=O), 1036 (–P–O–C–), 755 (–P–C–). ^{1}H-NMR ($CDCl_3$): *δ* 7.93 (2H, d, *J* = 8.0 Hz), 7.66 (2H, t, *J* = 12.0 Hz), 7.62 (1H, d, *J* = 8.0 Hz), 7.44 (1H, t, *J* = 12.0 Hz), 7.33(1H, m), 7.26 (1H, m), 5.89 (1H, s), 4.97 (1H, d, *J* = 12.0 Hz), 4.08–3.74 (4H, m), 2.33 (3H, s), 1.19 (3H, t, *J* = 12.0 Hz), 1.15 (3H, t, *J* = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): *δ* 147.67, 145.93, 142.91, 138.49, 137.51, 132.63, 131.46 (d, *Jc*-p = 64.0 Hz), 128.84 (d, *Jc*-p = 20.0 Hz), 126.21, 124.57 (d, *Jc*-p = 20.0 Hz), 121.63 (d, *Jc*-p = 192.0 Hz), 103.65, 63.44 (d, *Jc*-p = 20.0 Hz), 61.90 (d, *Jc*-p = 12.0 Hz), 44.66 (d, *Jc*-p = 240.0 Hz), 16.39, 12.64, 11.86. ^{31}P-NMR ($CDCl_3$): *δ* 21.78. MS (ESI): *m/z* 480.10 Anal. Calcd. for $C_{21}H_{23}ClN_3O_6P$: C, 52.56; H, 4.83; Cl, 7.39; N, 8.76; O, 20.01; P, 6.45. Found: C, 52.45; H, 4.74; Cl, 7.31; N, 8.66; O, 19.94; P, 6.36.

Diethyl((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(naphthalen-2-yl)methyl)phosphonate(**4m**) [24, 25] Light yellow solid; Yield: 93%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *ν* 3436 (–OH), 2935 (Ar–H), 1235 (–P=O), 1041 (–P–O–C–), 738 (–P–C–). ^{1}H-NMR ($CDCl_3$): *δ* 8.25 (1H, d, *J* = 8.0 Hz), 7.90 (3H, d, *J* = 7.0 Hz), 8.0 (2H, m), 7.64 (1H, t, *J* = 7.0 Hz), 7.57–7.43 (4H, m), 7.29–7.24 (1H, m), 5.25 (1H, d, *J* = 27.9 Hz), 5.80 (1H, s), 4.16–3.78 (4H, m), 2.42 (3H, s), 1.62 (3H, t, *J* = 12.0 Hz), 1.14 (3H, t, *J* = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): *δ* 151.76 (d, *Jc*-p = 54.70 Hz), 148.1 (d, *Jc*-p = 124.28 Hz), 139.34, 134.42, 132.36 (d, *Jc*-p = 48.0 Hz), 131.51 (d, *Jc*-p = 28.0 Hz), 129.16 (d, *Jc*-p = 28.0 Hz), 128.40 (d, *Jc*-p = 24.0 Hz), 126.66, 126.08 (d, *Jc*-p = 24.0 Hz), 125.9 (d, *Jc*-p = 32.0 Hz), 123.50, 122.96, 94.12 (d, *Jc*-p = 48.0 Hz), 64.5 (d, *Jc*-p = 54.0 Hz), 63.6 (d, *Jc*-p = 76.0 Hz), 42.60 (d, *Jc*-p = 137.0 Hz), 16.54, 13.79, 12.16. ^{31}P-NMR ($CDCl_3$): *δ* 21.16. MS (ESI): *m/z* 451.17 Anal. Calcd. for $C_{25}H_{27}N_2O_4P$: C, 66.66; H, 6.04; N, 6.22; O, 14.21; P, 6.88. Found: C, 66.58; H, 5.96; N, 6.13; O, 14.12; P, 6.79.

Diethyl(anthracen-9-yl(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate(**4n**) Brown solid; Yield: 94%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *ν* 3391 (-OH), 2986 (Ar–H), 1247 (–P=O), 1047 (–P–O–C–), 726 (–P–C–). ^{1}H-NMR ($CDCl_3$): 8.29 (1H, d, *J* = 8.0 Hz), 7.95 (2H, d, *J* = 8.0 Hz), 7.91 (2H, t, *J* = 12.0 Hz), 7.58 (2H, d, *J* = 8.0 Hz), 7.51 (2H, t, *J* = 12.0 Hz), 7.44-7.38 (5H, m), *δ* 5.82 (1H, s), 5.12 (1H, d, *J* = 8.0 Hz), 4.22–3.82 (4H, m), 2.64 (3H, s), 1.35 (3H, t, *J* = 12.0 Hz), 1.12 (3H, t, *J* = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): *δ* 145.86 (d, *Jc*-p = 58.0 Hz), 145.25 (d, *Jc*-p = 12.0 Hz), 138.16, 133.42, 130.48 (d, *Jc*-p = 24.0 Hz), 129.58 (d, *Jc*-p = 48.0 Hz), 129.16 (d, *Jc*-p = 48.0 Hz), 127.45 (d, *Jc*-p = 24.0 Hz), 126.90, 126.46 (d, *Jc*-p = 24.0 Hz), 124.86 (d, *Jc*-p = 36.0 Hz), 124.36 (d, *Jc*-p = 84.0 Hz), 124.19 (d, *Jc*-p = 24.0 Hz), 122.08, 98.42 (d, *Jc*-p = 76.0 Hz), 64.49 (d, *Jc*-p = 24.0 Hz), 63.46 (d, *Jc*-p = 24.0 Hz), 45.62 (d, *Jc*-p = 158.0 Hz), 16.51, 13.80, 12.14. ^{31}P-NMR ($CDCl_3$): *δ* 19.26. MS (ESI): *m/z* 501.30 Anal.Calcd. for $C_{29}H_{29}N_2O_4P$: C, 69.59; H, 5.84; N, 5.60; O, 12.79; P, 6.19. Found: C, 69.48; H, 5.76; N, 5.51; O, 12.70; P, 6.11.

Diethyl((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(pyren-1-yl)methyl)phosphonate(**4o**) Brown solid; Yield: 94%. M.p. > 300 °C. FT-IR (neat, cm^{-1}) *ν* 3386 (OH), 2998 (Ar–H), 1224 (–P=O), 1043 (–P–O–C–), 746 (–P–C–). ^{1}H-NMR ($CDCl_3$): 8.29 (1H, d, *J* = 8.0 Hz), 7.93 (2H, t, *J* = 12.0 Hz), 7.78 (2H, d, *J* = 8.0 Hz), 7.73 (1H, d, *J* = 8.0 Hz), 7.71-7.68 (4H, m), 7.62 (1H, t,

J = 12.0 Hz), 7.49 (2H, d, J = 8.0 Hz), 7.43 (1H, t, J = 12.0 Hz), 7.37–7.34 (1H, m), δ 5.88 (1H, s), 4.51 (1H, d, J = 8.0 Hz), 4.18–4.10 (2H, m), 4.06-3.80 (4H, m), 2.56 (3H, s), 1.24 (3H, t, J = 12.0 Hz), 1.12 (3H, t, J = 12.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 147.36 (d, Jc-p = 48.0 Hz), 145.52 (d, Jc-p = 57.0 Hz), 138.10, 135.21 (d, Jc-p = 120.0 Hz), 131.43, 130.52 (d, Jc-p = 54.0 Hz), 129.76 (d, Jc-p = 58.0 Hz), 129.44 (d, Jc-p = 28.0 Hz), 129.34 (d, Jc-p = 20.0 Hz), 128.26 (d, Jc-p = 58.0 Hz), 127.86 (d, Jc-p = 54.0 Hz), 127.64 (d, Jc-p = 28.0 Hz), 127.48, (d, Jc-p = 28.0 Hz), 126.45 (d, Jc-p = 48.0 Hz), 125.06 (d, Jc-p = 36.0 Hz), 122.62 (d, Jc-p = 54.0 Hz), 98.18 (d, Jc-p = 84.0 Hz), 64.6 (d, Jc-p = 24.0 Hz), 63.4 (d, Jc-p = 12.0 Hz), 48.6 (d, Jc-p = 154.0 Hz), 16.54, 13.86, 11.61. ^{31}P-NMR ($CDCl_3$): δ 22.16. MS (ESI): *m/z* 525.19 Anal. Calcd. for $C_{31}H_{29}N_2O_4P$: C, 70.98; H, 5.57; N, 5.34; O, 12.20; P, 5.90. Found: C, 70.91; H, 5.48; N, 5.22; O, 12.15; P, 5.82.

Anti-oxidant assay

The anti-oxidant activity of the title compounds (**4a–4o**) was evaluated by DPPH [33] and H_2O_2 [34] methods,. Ascorbic acid was used as standard control. The radical scavenging activity (RSA) was calculated by using the equation:

$$\%\text{RSA} = [(A_c - A_s)] \times 100$$

where A_c is the absorbance of the control and A_s is the absorbance of the tested sample. The anti-oxidant activity was also expressed as IC_{50}. The IC_{50} values were well defined as the concentration (in μg/mL) of the compounds at which formation of DPPH and H_2O_2 radicals was inhibited by 50%.

In vitro anticancer assay

The in vitro anticancer activity of the title compounds (**4a–o**) was tested against the proliferation of MCF-7 (breast) DU-145 (prostate) and A-549 (lung cancer) cell lines by performing a sulforhodamine-B (SRB) assay [35]. The cell line of interest was seeded in disinfected flat-bottomed 96-well plate (5000 cells/100 μL) in a medium containing 10% fetal bovine serum and antibiotics (penicillin and streptomycin). After incubation for 18–20 h in an incubator continuously supplied with 5% CO_2 to ensure appropriate adherence of the cells to the surface bottom of the wells, the cells were treated with the compounds or the reference standard doxorubicin. To treat cells, working dilutions of concentrations of the compounds were prepared, of which a 2-μL aliquot was added to every well, thereby creating the final concentration of the compounds of 0–100 μM. Each compound was tested in triplicate and the cytotoxicity was determined as the average of that triplicate. DMSO and doxorubicin (as standard control anti-cancer drug) were taken as vehicle and positive controls respectively. Further, the cells were allowed to grow for another 48 h in an incubator maintained at 37 °C with a constant supply of 5% CO_2. The plates were then air-dried and 100 μL of 10 mM Tris Base was added to each well to solubilize the SRB before reading the absorbance using Perkin-Elmer

Multimode Reader at 510 nm. The amount of absorbance is directly relative to cell growth and is thus used to calculate the IC_{50} values. In this study, for initial screening, three types of cancer cell lines, i.e. human breast cancer (MCF-7), lung cancer (A-549), and prostate cancer (DU-145) cell lines were tested for the cytotoxic effect of the series of compounds.

Results and discussion

Chemistry

A strategic approach has been made for the C-P bond formation in the one-pot synthesis of pyrazolylphosphonates (**4a–o**) by the reaction of 3-methyl-1-phenyl-5-pyrazolone (**1**) with various aryl aldehydes (**2a–o**) and diethyl phosphite (**3**) under green conditions using β-CD as a catalyst, which offered good to excellent yields after a simple work-up procedure.

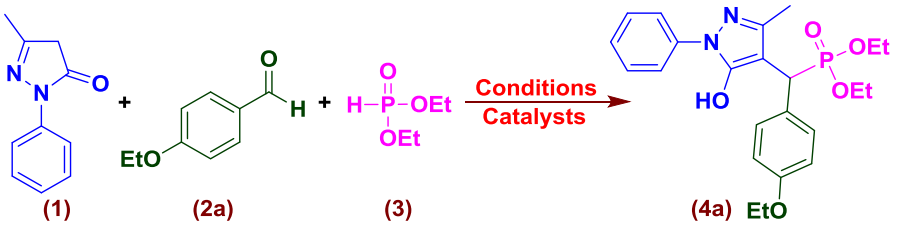
In order to determine the best experimental conditions, a model reaction has been carried out by taking 3-methyl-1-phenyl-5-pyrazolone (**1**; 1 mmol), 4-ethoxybenzaldehyde (**2a**; 1.2 mmol) and diethyl phosphite (**3**; 2 mmol) for the synthesis of diethyl((5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)(4-ethoxyphenyl)methyl)phosphonate (**4a**). Initially, we have run the reaction under room temperature (r.t.) and solvent-free conditions without a catalyst which resulted in no reaction, even after 24 h (Table 1, entry 1). Then, we used different catalysts such as $FeCl_3$, $InCl_2$, $ZnCl_2$, $ZnBr_2$, $CeCl_3.7H_2O$, $BF_3.SiO_2$, Amberlyst-15, p-TSA, PS-PTSA, TMG, DABCO, DBU and β-CD (Table 1, entries 2–15). Among all of them, β-CD in 10 mol% showed better catalytic activity; the reaction proceeded very smoothly and gave the product **4a** in 96% yield (Table 1, entry 15).

The reaction proceeded smoothly in solvent-free conditions. However, to study the effect of solvent on the reaction, various polar solvents like methanol, ethanol, isopropanol, tetrahydrofuran, and acetonitrile were tried. The reaction in methanol, ethanol and isopropanol (Table 2, entries 1–3) decreased the product yield, but the rate of reaction was increased due to the solubility factor. In THF and acetonitrile (Table 2, entries 4, 5) to obtain 88 and 73% yields, the reaction has to be run for 4 and 3 h, respectively, owing to the aprotic polar nature of the solvent. When the reaction was run in non-polar solvents such as toluene, CH_2Cl_2, and $CHCl_3$ (Table 2, entries 6–8), both the rate of the reaction and product yields were decreased.

To determine the optimum catalyst β-CD loading, model reactions were carried out using 1, 3, 5, 10, and 15 mol% (Table 2, entries 9–13) of β-CD at room temperature under solvent-free conditions and obtained the product yields of 60, 73, 85, 96 and 96%, respectively. Increasing the amount of catalyst beyond 10 mol% had no additional effect either on the reaction rate or the product yield.

After establishing the optimum reaction conditions, we investigated the scope of the reaction by condensing 3-methyl-1-phenyl-2-pyrazoline-5-one (**1**) with various commercially available substituted aryl aldehydes having different electronically activating or deactivating substituents (**2a–o**) and diethyl phosphite (**3**) to form

Table 1 Effect of the various catalysts on the synthesis of diethyl((5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)(4-ethoxyphenyl)methyl)phosphonate (**4a**)



Entry	Catalyst (mol%)	Time (min)	Yield[a] (%)
1.	No catalyst	24 h	Nr[b]
2.	$FeCl_3$ (10)	30	32
3.	$InCl_3$ (10)	30	38
4.	$ZnCl_2$ (10)	30	35
5.	$ZnBr_2$ (10)	30	43
6.	BF_3-OEt_2 (10)	30	54
7.	$CeCl_3 \cdot 7H_2O$ (10)	30	55
8.	$BF_3 \cdot SiO_2$ (10)	30	62
9.	Amberlyst 15 (10)	30	69
10.	p-TSA (10)	30	75
11.	PS-PTSA (10)	30	82
12.	TMG (10)	30	85
13.	DABCO (10)	30	87
14.	DBU (10)	30	90
15.	β-CD (10)	30	96

Reaction of 3-methyl-1-phenyl-5-pyrazolone (1 mmol), 4-ethoxybenzaldehyde (1.2 mmol) and diethyl phosphite (2 mmol) using various catalysts along with *β*-CD in different concentrations under r.t. and neat conditions

[a]Isolated yield

[b]No reaction

corresponding pyrazolylphosphonates (**4a–o**). The details of the physical data, such as yield and melting points, are illustrated in Table 3.

The plausible reaction sequences taking place in the synthesis of pyrazolylphosphonats is shown in Scheme 2. *β*-CD favors the Knoevenagel condensation through activation of the carbonyl group of aldehyde (**a**) via hydrogen bonding (supramolecular interaction) [32] and form intermediate (**b**), which can react with 3-methyl-1-phenyl-1*H*-pyrazol-5-ol (**d**) (enol form of 3-methyl-1-phenyl-2-pyrazoline-5-one (**c**)) to form intermediate (**e**) which on dehydration forms (**f**). Phosphorylation reaction of (**f**) with diethyl phosphite leads to the formation of corresponding pyrazolylphosphonate (**g**).

Table 2 Optimization of reaction, conditions on solvent and catalyst loading for the synthesis of **4a**

Entry	Catalyst (mol %)	Solvent	Time (h)	Yield (%)[a]
1.	β-CD (10)	Methanol	2	86
2.	β-CD (10)	Ethanol	5	87
3.	β-CD (10)	IPA	6	85
4.	β-CD (10)	THF	4	88
5.	β-CD (10)	CH_3CN	3	73
6.	β-CD (10)	Toluene	18	55
7.	β-CD (10)	CH_2Cl_2	24	67
8.	β-CD (10)	$CHCl_3$	15	60
9.	β-CD (1)	Solvent-free	0.5	60
10.	β-CD (3)	Solvent-free	0.5	73
11.	β-CD (5)	Solvent-free	0.5	85
12.	β-CD (10)	Solvent-free	0.5	96
13.	β-CD (15)	Solvent-free	0.5	96

Reaction conditions: 3-methyl-1-phenyl-5-pyrazolone (1.0 mmol), 4-ethoyybenzaldehyde (1.2 mmol), and diethylphosphite (2.0 mmol) in the presence of β-CD

[a]Yield of the isolated product. Solvent-free and r.t. conditions

The title compounds (**4a–o**) were fully characterized by physical and spectral (FT-IR, NMR, and mass) data. In the FT-IR spectra of the title compounds, the following bands were detected: (1) –OH stretching vibrations at 3442–3364 cm^{-1}; (2) –P=O stretching vibrations at 1254–1180 cm^{-1}; (3) –P–O–C stretching vibrations at 1048–1006 cm^{-1}; and (4) P–C stretching vibrations at 780–720 cm^{-1}. In the 400 MHz ^{1}H–NMR spectra of **4a–o** (in $CDCl_3$), the following signals were detected: (1) the chemical shifts in the region of 8.24–6.48 ppm are due to aromatic protons; (2) a singlet at δ 5.96–5.62 confirmed the –OH proton and the doublet at 5.64–4.46 ppm corresponds to H–C–P proton; (3) the multiplet in the region of 3.98–3.82 ppm and multiplet in the region of 3.56–3.32 ppm are due to the O–CH_2–CH_3 protons; (4) a singlet at 2.52–2.36 ppm is due to CH_3 protons; and (5) a triplet at 1.38–1.18 ppm and a triplet at 1.24–1.12 ppm are due to the O–CH_2–CH_3 protons. ^{13}C–NMR (100 MHz, $CDCl_3$, ppm) reveals that the chemical shifts in the region of 184.5–101.3 ppm are assigned to carbons of aromatic ring, the signals in the region of 64.8–58.6, 59.2–48.8 and 16.3–16.2 ppm confirmed the –O–CH_2–CH_3, H–C–P and –O–CH_2–CH_3 carbons, and at 24.6–21.4 and 12.7–11.8 ppm confirmed the –CH_3 carbon. The ^{31}P NMR (165.9 MHz, $CDCl_3$ and ppm) chemical shifts of the title compounds appeared in the range from 18.7 to 24.6 ppm.

Pharmacology

In vitro anti-oxidant activity

The synthesized pyrazolylphosphonates (**4a–o**) were screened for their free radical scavenging activity by the DPPH [33] and H_2O_2 [34] methods. Ascorbic acid was used as standard control (Table 4). A lower IC_{50} value is associated with a higher radical scavenging activity. The DPPH and H_2O_2 radical scavenging activities of the

Table 3 Synthesis of pyrazolylphosphonates (**4a–o**) on *β*-CD catalyst by r.t. and neat conditions via Scheme 1

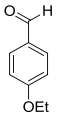
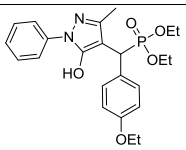
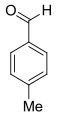
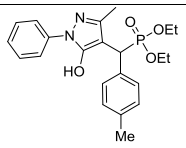
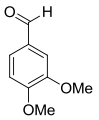
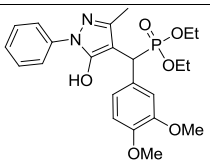
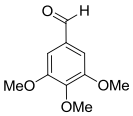
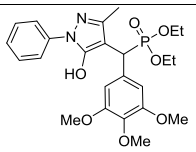
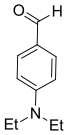
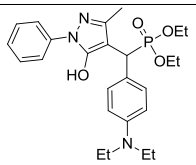
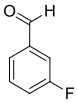
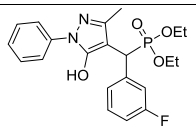
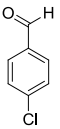
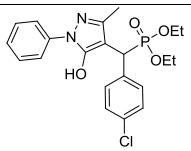
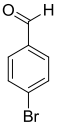
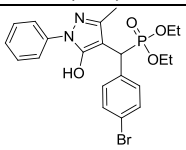
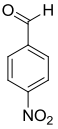
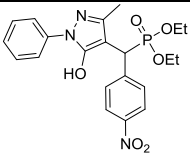
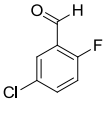
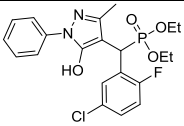
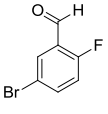
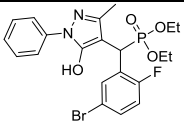
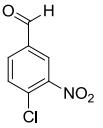
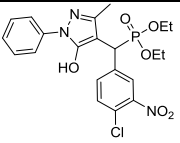
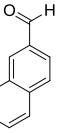
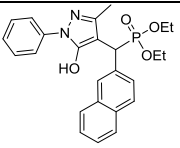
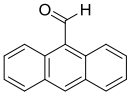
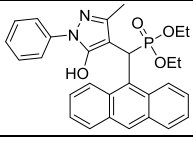
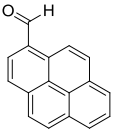
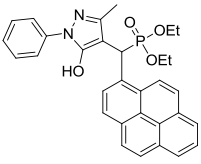
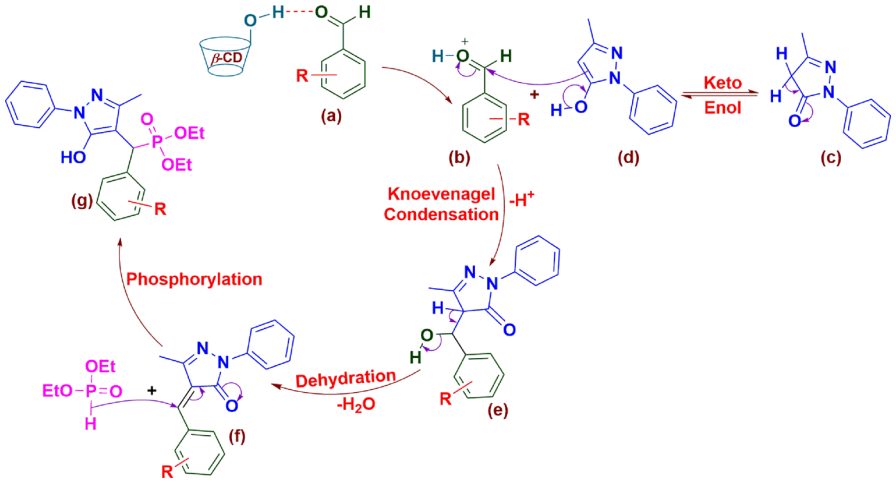
S. No.	Structures of aryl aldehydes **(2a–o)**	Structures of products **(4a–o)**	Time (Min)	Yield[a] (%)	Melting Point (˚C)
1			30	96	> 300
2			30	96	> 300 [24]
3			30	95	> 300
4			30	94	> 300
5			30	96	> 300
6			30	91	> 300
7			30	92	> 300 [23]

Table 3 continued

S. No.	Structures of aryl aldehydes **(2a–o)**	Structures of products **(4a–o)**	Time (Min)	Yield[a] (%)	Melting Point (°C)
8			30	92	> 300
9			30	91	> 300 [24,25]
10			30	90	> 300
11			30	89	> 300
12			30	88	> 300
13			30	93	> 300 [24,25]
14			30	94	> 300
15			30	94	> 300

Reaction conditions: 3-methyl-1-phenyl-2-pyrazoline-5-one (1.0 mmol), various substituted aryl aldehydes (1.2 mmol), and diethyl phosphite (2.0 mmol) in the presence of 10 mol% of β-CD as catalyst at solvent-free and r.t. conditions in 30 min

[a]Isolated yields



Scheme 2 Plausible mechanism for the catalytic activity of *β*-CD in the formation of pyrazolylphosphonates (**4a–o**)

title compounds (**4a–o**) are shown in Table 4. Compounds **4g**, **4i**, **4h** and **4f** exhibited the lowest anti-oxidant activities, compared to the IC_{50} of ascorbic acid used as standard. While nearly the same activities were revealed in the compounds **4c** and **4d** compared to the IC_{50} of ascorbic acid. The compounds **4m**, **4n**, **4o**, **4j**, **4k** and **4l** showed moderate to good anti-oxidant activity and the compounds **4e**, **4b** and **4a** showed potent anti-oxidant activity compared to the IC_{50} of ascorbic acid.

In vitro anticancer activity

The synthesized pyrazolylphosphonates (**4a–o**) were subjected to the well-known SRB cytotoxic assay [35] against human breast (MCF-7), prostate (DU-145), and lung (A-459) cancer cell lines to investigate the effectiveness of the in vitro cell cytotoxic properties (Table 5). All the data were expressed as IC_{50} values. The obtained data revealed that the compounds **4o**, **4n** and **4m** have excellent cell growth inhibitory effects on MCF-7, DU-145 and A-549 cell lines with IC_{50} values compared to the IC_{50} of the doxorubicin standard used. While compounds **4l**, **4f**, **4h**, **4g** and **4i** showed moderate cytotoxic activity, the remaining compounds, **4k**, **4j**, **4b**, **4c**, **4b**, **4a** and **4e**, could not show effective cytotoxic activity on the three cell lines.

Structure activity relationship (SAR) studies

Generally, the presence of electron-donating substituents such as *N*-alkyl, alkoxy and alkyl groups enhances the anti-oxidant property while electron-withdrawing aryl and halogen groups suppress the DPPH and H_2O_2 radical scavenging ability. The structure activity relationship (SAR) studies regarding anti-oxidant activity of the synthesized compounds with electron donor substituents like **4e**, **4d**, **4c**, **4b** and **4a** bearing 4-(diethylamino)phenyl, 3,4,5-trimethoxyphenyl, 3,4-dimethoxyphenyl,

Table 4 Free radical scavenging activity of pyrazolylphosphonates (**4a–o)**

S. no.	Tested samples	DPPH radical scavenging activity IC_{50} (μg/mL)	Hydrogen peroxide scavenging activity IC_{50} (μg/mL)
1.	**4a**	12.35	23.55
2.	**4b**	11.39	22.76
3.	**4c**	13.57	24.47
4.	**4d**	13.88	24.88
5.	**4e**	10.24	19.57
6.	**4f**	41.26	53.18
7.	**4g**	28.81	40.81
8.	**4h**	38.67	48.67
9.	**4i**	32.93	42.93
10.	**4j**	19.85	30.64
11.	**4k**	20.32	33.12
12.	**4l**	21.45	29.25
13.	**4m**	15.77	27.47
14.	**4n**	18.63	26.83
15.	**4o**	19.24	27.24
16.	Ascorbic acid	13.67	24.52

Table 5 In vitro anticancer activity of pyrazolylphosphonates **(4a–o)**

S. no.	Tested samples	MCF-7	DU-145	A-549
IC_{50} (μg/mL)				
1.	**4a**	46.885	36.673	42.959
2.	**4b**	12.634	10.187	10.892
3.	**4c**	32.672	26.773	34.515
4.	**4d**	29.387	18.166	26.134
5.	**4e**	52.632	38.401	48.964
6.	**4f**	13.855	10.869	11.863
7.	**4g**	17.678	13.144	16.514
8.	**4h**	15.284	11.824	13.039
9.	**4i**	20.987	15.543	19.120
9.	**4j**	11.673	9.316	10.230
10.	**4k**	10.453	8.861	9.646
11.	**4l**	26.423	16.826	20.763
12.	**4m**	9.867	9.839	8.113
13.	**4n**	9.187	7.672	6.483
14.	**4o**	7.854	6.753	5.967
15.	Doxorubicin	9.652	7.114	8.340

4-ethylphenyl and 4-methyl phenyl groups were found to be the most active derivatives. Compounds with electron withdrawing groups **4g**, **4i**, **4h**, **4f**, **4j** and **4k** showed moderate radical scavenging activity, whereas compounds **4m**, **4n** and **4o** with poly aromatics like naphthalene, anthracene and pyrene ring systems revealed less activity.

SAR studies showed that the anticancer activity increased with the increase of the electron-withdrawing nature of groups on the phenyl ring. The compounds having poly aromatics like naphthalene, anthracene and pyrene ring systems (**4m**, **4n** and **4o**) showed pronounced anticancer activity, followed by the compounds having halo and nitro substituted phenyl groups (**4g**, **4i**, **4h**, **4f**, **4j**, **4k** and **4l**). the compounds with electron donor substituents like 4-(diethylamino) phenyl, 3,4,5-trimethoxyphenyl, 3,4-dimethoxy phenyl, 4-ethylphenyl and 4-methylphenyl groups (**4e**, **4d**, **4c**, **4b** and **4a**) were found to be the least active anticancer agents.

Conclusion

In summary, we have demonstrated an efficient and eco-friendly protocol for the synthesis of pyrazolylphosphonates through the β-CD catalyzed reaction of pyrazolone with aryl aldehydes and diethyl phosphite. This new method is endowed with green reaction conditions such as low cost, use of non-toxic catalyst, solvent-free medium, easy work-up process and good yields. The title compounds were screened for anti-oxidant activity by DPPH and H_2O_2 radical scavenging assay and anticancer activity against breast cancer (MCF-7), prostate cancer (DU-145) and lung cancer (A-549) cell lines with sulfarodamine-B assay. The compounds **4e, 4d, 4c, 4b** and **4a** showed promising anti-oxidant activity and the compounds **4m, 4n** and **4o** showed significant anticancer activity.

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